Prospective Study of Serum Selenium Levels and Incident Esophageal and Gastric Cancers

Steven D. Mark, You-Lin Qiao, Sanford M. Dawsey, Yan-Ping Wu, Hormuzd Katki, Elaine W. Gunter, Joseph F. Fraumeni, Jr., William J. Blot, Zhi-Wei Dong, Phillip R. Taylor

Background: **From March 1986 through May 1991, we conducted a randomized nutritional intervention trial, the General Population Trial, in Linxian, China, a region with epidemic rates of squamous esophageal and adenomatous gastric cardia cancers. We found that participants who received selenium, β-carotene, and vitamin E had significantly lower cancer mortality rates than those who did not. In the current study, we examined the relationship between selenium levels measured in pretrial (1985) sera from participants and the subsequent risk of developing squamous esophageal, gastric cardia, and gastric non-cardia cancers during the trial.** ***Methods:*** **This study was designed and analyzed in accord with a stratified case–cohort sampling scheme, with the six strata defined by sex and three age categories. We measured serum selenium levels in 590 case subjects with esophageal cancer, 402 with gastric cardia cancers, and 87 with gastric non-cardia cancers as well as in 1062 control subjects. Relative risks (RRs), absolute risks, and population attributable risk for cancers were estimated on the basis of the Cox proportional hazards models. All statistical tests are two-sided.** ***Results:*** **We found highly significant inverse associations of serum selenium levels with the incidence of esophageal (*P* for trend $<10^{-4}$) and gastric cardia (*P* for trend $<10^{-6}$) cancers. The RR and 95% confidence interval (CI) for comparison of highest to lowest quartile of serum selenium was 0.56 (95% CI = 0.44–0.71) for esophageal cancer and 0.47 (95% CI = 0.33–0.65) for gastric cardia cancer. The population proportion of these cancers that is attributable to low selenium levels was 26.4% (95% CI = 14.45–38.36). We found no evidence for a gradient of serum selenium associated with incidence of gastric non-cardia cancer (*P* for trend = .96), with an RR of 1.07 (95% CI = 0.55–2.08) for the highest to lowest quartile of serum selenium.** ***Conclusions:*** **Our study supports findings from previous prospective studies and randomized trials that variations in selenium levels affect the incidence of certain cancers. In the United States, where intervention trials of selenium are in the planning stages, consideration should be given to including populations at high risk for squamous esophageal and gastric cardia cancers. [J Natl Cancer Inst 2000;92:1753–63]**

Selenium, an essential trace element for humans, is necessary for the formation and function of at least 13 proteins *(1).* Both the enzymatic function of these proteins and the nonenzymatic activity of selenium metabolites have been implicated in several tissue repair and cell-regulatory pathways important in carcinogenesis. These pathways include the repair and prevention of oxidative damage, intracellular signaling, activation of thyroid hormone, regulation of immune response, and P53-independent apoptosis *(1–5).* Animal experiments *(2,3)* have found that increases in the dietary intake of selenium lead to reductions in the incidence of various cancers. In recent years, several prospective cohort studies and randomized intervention trials have suggested a link between selenium levels and human carcinogenesis.

In three large, randomized, placebo-controlled trials, selenium supplementation was given either alone *(6)* or with other elements *(7–9).* Clark et al. *(6)* carried out a study in the United States on 1312 subjects with an average follow-up of 4.5 years. That study was designed to test whether selenium supplementation could reduce the incidence of nonmelanoma skin cancers in high-risk individuals. Although no benefit was found for skin cancers, the group receiving the supplement had statistically significant reductions of approximately 40% and 50% in overall cancer incidence and cancer mortality, respectively. The other two studies, the larger of which forms the cohort investigated in this article, were conducted in Linxian, China, a region whose occupants had poor nutrition as well as rates of squamous esophageal/adenomatous gastric cardia cancers of more than 400 per 100 000 person-years *(10)* [approximately 100 times the rates of U.S. whites *(10)*]. The primary objective of both Linxian studies was to test whether nutritional supplementation would reduce the rates of overall mortality and the mortality from and incidence of esophageal and gastric cardia cancers. In the smaller of these two studies, a multivitamin containing selenium was randomly assigned to 3318 people with pre-existing esophageal dysplasia. At the end of the 6-year intervention, the group receiving the supplement had statistically nonsignificant (at the .05 level) reductions of 7% for total mortality and 8% for esophageal/gastric cardia cancer mortality. (Esophageal/gastric cardia cancers denote the combined total of squamous esophageal and adenomatous gastric cardia cancers.) The group receiving the supplement had a statistically significant increase of 25% *(11)* in reversion to normal cytology. The larger trial was referred to as the General Population Trial, since 29 584 participants were sampled from the general population of Linxian. The trial tested four different combinations of nutrient supplements for 5.25 years. The group receiving the supplement with selenium, β-carotene, and vitamin E had a statistically significant reduc-

Affiliations of authors: S. D. Mark, H. Katki, J. F. Fraumeni, Jr. (Division of Cancer Epidemiology and Genetics), S. M. Dawsey, P. R. Taylor (Division of Clinical Science), National Cancer Institute, Bethesda, MD; Y.-L. Qiao, Y.-P. Wu, Z.-W. Dong, Department of Epidemiology, Cancer Institute, Chinese Academy of Medical Sciences, Beijing, China; E. W. Gunter, National Health and Nutrition Examination Survey Laboratory, Centers for Disease Control and Prevention, Atlanta, GA; W. J. Blot, International Epidemiology Institute, Rockville, MD.

Correspondence to: Steven D. Mark, M.D., Sc.D., National Institutes of Health, 6120 Executive Blvd., Rm. 8036 MSC 7244, Rockville, MD 20852-4910 (e-mail: sm7v@.nih.gov).

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tion of 9% in all-cause mortality and 13% in cancer mortality *(8).* The mortality and incidence rates for esophageal/gastric cardia cancers were reduced by 10% and 6%, respectively *(8).* The subjects in the General Population Trial comprise the cohort of individuals in our current serologic study of selenium.

In a large number of nonrandomized, prospective cohort studies *(12–35),* selenium has been measured from stored biologic samples, usually plasma or serum, obtained from healthy individuals who were subsequently monitored for cancer and death. Typically, these studies accrued only a small number of cancer cases, making it difficult to obtain precise estimates of the relationship of selenium levels with the occurrence of a specific cancer. The largest amount of data exists for lung cancer *(17–19,22,24,28–31),* where the magnitude of the inverse association with low selenium levels was dependent on the range of serum selenium levels in the population under study. Populations with average serum selenium values in the range of 50–80 μg/L generally showed a decrease in lung cancer with increasing selenium levels, whereas populations with mean selenium values greater than 100 μg/L showed either no association or an association restricted to subjects at the lowest end of the population distribution.

Other than one study reporting on nine cases of esophageal cancer *(12),* there are no prospective studies evaluating the association of serum selenium levels with the occurrence of esophageal or gastric cardia cancers, although several studies *(13–18)* have investigated stomach cancer in general. One cohort study from Finland, where serum values were generally low *(13,14),* showed a decreased risk of stomach cancer with increasing selenium levels. Most other studies found either a weak association *(16)* or no association *(15,17,18).*

The primary goal of our study was to evaluate the relationship between levels of selenium in serum drawn before the beginning of supplementation in the General Population Trial with the subsequent development of 590 cases of squamous esophageal cancers, 402 cases of gastric cardia cancers, and 87 cases of gastric non-cardia cancers. We also set out to test whether the effects of selenium varied in subgroups defined by sex or smoking, as previously suggested *(13,14,16,20,21,23,24),* and whether the effects differed among those who received selenium supplementation in the trial versus those who did not. Finally, if a relationship was found between low selenium levels and higher cancer rates, we wanted to estimate the potential impact that correcting the selenium deficit would have on reducing the epidemic rates of cancer in this population.

Subjects and Methods

Cohort Population

The subjects in this study were selected from the cohort of all participants in the General Population Trial of Linxian. Elsewhere we have described in detail the design, choice of intervention agents, methods of conduct, and primary end-point analyses of the trial *(7,8).* In brief, the participants were 29 584 healthy adults aged 40–69 years from four Linxian communes. In the spring of 1985, 1 year before the start of the intervention, each participant was interviewed, was given a brief physical examination, and had 10 mL of blood drawn. Intervention began in March 1986 and continued through May 1991. In accord with a partial factorial design, the participants were randomly assigned to receive either a vitamin–mineral combination or a placebo. In total, four different vitamin–mineral combinations were tested: factor A (10 000 IU of vitamin A and 45 mg of zinc oxide), factor B (52 mg of riboflavin and 40 mg of niacin), factor C (180 mg of ascorbic acid and 30 μg of molybdenum), and factor D (50 μg of yeast selenium, 15 mg of β-carotene, and 30 mg of α-tocopherol). Village doctors ascertained mortality among trial participants through monthly follow-up. Diagnoses of cancer were ascertained through local commune and county hospitals and were supplemented by a study team that provided clinical and diagnostic services, including endoscopy, for patients with symptoms suggestive of esophageal or stomach cancer. A panel of U.S. and Chinese experts reviewed the diagnostic material for 90% of the cancer cases in this study. For anatomic localization of gastric adenocarcinomas, cancers were defined as cardia cancers if they were in the most proximal 3 cm of the stomach and as non-cardia cancers if they originated outside this region. Ninety-five percent of the anatomic localizations were made with the use of endoscopy, surgery, and/or x-rays. For cancer cases without diagnostic material and for deaths due to causes other than cancer, senior Chinese diagnosticians conducted reviews. At the end of trial in May 1991, interviews and brief physical examinations were conducted. In addition, 3421 asymptomatic individuals underwent a screening cytology or endoscopy. We obtained written informed consent from each participant before trial enrollment. Throughout the trial, human subject protection procedures were followed in accord with those prescribed by the U.S. National Institutes of Health and the Chinese Academy of Medical Sciences.

Selection of Study Participants for Serum Selenium Measurement

We used a stratified case–cohort design *(36,37)* to select individuals for selenium measurement from the cohort of all participants in the General Population Trial. By the end of the trial, there were a total of 640 incident esophageal squamous cell carcinomas, 435 incident gastric cardia adenocarcinomas, and 104 gastric non-cardia adenocarcinomas *(8).* We subsequently refer to these cancers without any additional histologic designation as esophageal cancer, gastric cardia cancer, and gastric non-cardia cancer, respectively. We denote the combined total of squamous esophageal and gastric cardia cancers as esophageal/gastric cardia cancer. Overall, 92% of the case subjects had adequate serum for selenium measurement. This amounted to 590 esophageal, 402 gastric cardia, and 87 gastric non-cardia cancer subjects (*see* Table 1). In addition, we measured selenium levels from a stratified random sample of all trial participants. The six strata were defined by sex and by the following three age categories at the start of the intervention: 1) 50 years old or younger, 2) older than 50 years to 60 years old, and 3) older than 60 years. A sufficient number of control subjects were drawn from each stratum to achieve a ratio of approximately 1 : 1 of control subjects to case subjects with incident esophageal/gastric cardia cancers. The lowest within-strata ratios of control subjects to case subjects for the incident site-specific cancers ranged from 1.5 to 2.2 (*see* Table 1). Overall, we measured selenium levels in 1062 control subjects and 1079 case subjects (*see* Table 1).

Laboratory Analyses

One year before the nutritional supplementation was started, 10 mL of blood was collected from each trial participant at the village examination centers. The blood specimens were stored on wet ice for the 3–6 hours required to transport the samples by van to the central Linxian field station. There, the serum was separated by standard centrifugation procedure, pipetted into vials, and immediately stored at −45 °C. After 3–4 days in storage, the specimens were shipped on dry ice to Beijing, China, where they were stored at −85 °C. In August 1996, the sera were transported on dry ice to the National Cancer Institute (NCI), Bethesda, MD. The specimens arrived frozen and were stored at −70 °C at the NCI–Frederick Cancer Research and Development Center repository, Frederick, MD. Samples were thawed, pipetted into aliquots in a laminar flow hood, and then immediately refrozen to −70 °C. The samples were shipped on dry ice to the National Health and Nutrition Examination Survey (NHANES) Laboratory, Centers for Disease Control and Prevention, Atlanta, GA, in March and July 1997. Serum specimens were analyzed for selenium by graphite furnace atomic absorption spectrophotometry, with Zeeman background correction, with the use of the established NHANES III method *(38).* Quality control for the serum selenium determinations was established by replicate analysis of three in-house serum pools, whose target values had been verified against National Institute of Standards and Technology (NIST) Reference Materials (SRMs) 909 and 909B (Gaithersburg, MD), as well as periodic measurement of the NIST SRMs themselves. An additional "ultra-low" pool was created by dilution of the existing low pool 1 : 2 with saline to obtain values in the same concentration range of that of the specimens in this study. All samples analyzed were well above the lower

detection limit of 8 μg/L. For more details on laboratory protocols and internal quality control for the NHANES Laboratory, *see* Gunter et al. *(39).*

The samples were shipped and analyzed in a sequence designed to minimize the possible bias in the estimation of cancer risk that could be introduced if selenium measurement varied by time or batch. Within every group of 10 samples, a sample from a case subject was always accompanied by a sample from a control subject from the same sex–age stratum. Case subjects with each of the three cancer types and each of the six strata were mixed throughout. For assessment of assay reliability, one of 10 samples was a quality-control sample of serum obtained from processing pooled whole blood drawn in 1996 from three residents of Linxian. Laboratory personnel were unaware of the case subject–control subject status and of the existence of these quality-control samples. The selenium measurements were made in 49 batches on 49 different days. A total of 245 blinded quality-control specimens were measured, with most batches containing four to six of these quality-control samples. The mean value of the blinded quality-control samples was 53.1 μg/L, with a standard deviation (SD) of 5.9 μg/L. To detect variation in the measurement procedure over time, we examined scatter plots, generalized additive models, and auto-regressive models of the quality-control selenium values versus time *(40).* We found no indication that the selenium measurements varied with time. We used variance component models *(40,41)* to estimate within- and between-batch variation. We estimated the coefficient of reliability *(41)* as 78%. The coefficient of reliability is equal to 1 – intraclass correlation coefficient and measures the percentage of measurement variability not attributable to the effect of differences between batches.

Statistical Analysis

To graphically examine the shape of the selenium distribution in the General Population Trial cohort, we used histograms, quantile–quantile plots, density plots, and plots of residuals from regressions of selenium on age and sex. Neither graphical displays nor the residual plots revealed deviations from normality, outliers, variance heteroscedasticity, or observations with high influence. The raw data were superior to the log-transformed data in these regards.

The mean values of serum selenium and quantiles (*see* Tables 2 and 3) as well as tests for differences in the means within subgroups were calculated with the use of the known sampling weights for each individual in the serum study *(42).* Thus, for example, the means and quantiles in Table 2 are estimates of the means and quantiles of the entire General Population Trial cohort and not, as is generally the case, the means and quantiles of those who never develop the cancers under study. All *P* values listed are for two-sided tests.

We measured the time to cancer as time since March 1986 (start of the intervention), as opposed to time since blood collection (March 1985). Individuals who developed cancer in this 1-year interval were excluded from the General Population Trial and from this analysis. When analyzing cancers of a specific site, we treated persons with cancers at other sites as censored at the time of cancer occurrence. We estimated relative risks (RRs) and 95% confidence intervals (CIs) by using the case–cohort estimator *(36,37,43)* for the Cox proportional hazards models. All estimates came from models stratified on the six sex–age sampling strata. Within each stratum, an additional stratum-specific age term for continuous age was used to adjust for variation of age within stratum. Nested models were compared with the use of score tests. To examine the assumption that RR was time invariant, we used both graphical means and time-dependent models (*see* Table 5). We performed a sensitivity analysis of all RRs presented by deleting the upper and lower most extreme 1% of selenium values. The RRs remained unchanged up to three significant digits.

To estimate age- and sex-adjusted absolute risk (cumulative incidence), we adapted methods of survival estimation developed for Cox proportional hazards analyses of a full cohort *(44)* to the stratified case–cohort sampling that we used. A manuscript describing the exact procedures and the computer code implementing these procedures is currently in preparation (Mark SD, Katki H). We calculated the cumulative incidence by subtracting the survival probability from unity. Designating individuals in the lower three quartiles of serum selenium to be the "exposed" population (E^+) and individuals in the highest quartile to be the "unexposed" (E^-) and defining D^+ to be the occurrence of the cancer(s) under study, we calculated the population attributable risk *(45)* based on the formula

$$\frac{Pr[E^+] \times (Pr[D^+ \mid E^+] - Pr[D^+ \mid E^-])}{Pr[D^+]},$$

where, for instance, $Pr[D^+ \mid E^+]$ is the probability of disease among the exposed. This quantity has also been called the etiologic fraction *(46).* We obtained the variance of our population attributable risk estimates by standard application of the delta method to the estimates of the absolute risk.

For all RRs and absolute risks, we examined three different selenium metrics. Individuals were classified by their actual selenium measurement (selenium per 11.0 μg/L, the approximate size of the central quartiles: [82.1 – 59.8]/2 = 11.15, rounded to 11.0 μg/L; *see* Table 2) by quartiles of selenium, allowing a separate effect for each quartile, and by quartile of selenium using the ordinal value of the quartile as a continuous measure. For both of the continuous measures, we tested deviations from log linearity by adding quadratic terms. For all three metrics, we tested for a "threshold effect" by including a separate parameter for those in the lowest decile of selenium. Since all three methods gave indistinguishable dose–response curves, we present estimates from only the first and second. To account for the possible differences in the biologic behavior between cancers detected by the death of the subject versus cancers detected while the subject was alive, we give separate effect estimates for the end point for cancer incidence (all cancers, both fatal and nonfatal) and cancer mortality (restricted to fatal cancers) for all estimates of the main effect of selenium on cancer (*see* Tables 3, 4, and 6). For interaction effects (*see* Table 5), we present only the results for incident cancers.

Results

Table 1 shows the counts of the number of case subjects and control subjects in each of the six age–sex sampling strata, as

Table 1. Mean age (standard deviation [SD]) for case and control subjects in the General Population Trial, Linxian, China, and number of site-specific incident and fatal cancers: overall and stratified by sex and age

		Case subjects					
		Esophageal cancer		Gastric cardia cancer		Gastric non-cardia cancer	
	Control subjects	Incidence	Death	Incidence	Death	Incidence	Death
Combined over sex–age strata							
Mean age (SD), y	56.35 (0.25)	56.41 (0.33)	58.03 (0.44)	57.26 (0.36)	58.71 (0.45)	58.44 (0.79)	59.10 (0.90)
Total No. of females and males	1062	590	332	402	232	87	68
Females							
Mean age (SD), y	54.99 (0.26)	55.21 (0.48)	57.14 (0.69)	56.38 (0.57)	57.29 (0.70)	55.10 (1.69)	56.42 (2.14)
≤50	129	88	35	36	19	6	3
50–60	207	123	55	77	49	10	6
>60	146	93	65	50	36	5	5
Total No. of females	482	304	155	163	104	21	14
Males							
Mean age (SD), y	57.47 (0.23)	57.67 (0.45)	58.82 (0.55)	57.86 (0.46)	59.86 (0.57)	59.50 (0.86)	59.80 (0.98)
≤50	88	52	22	37	11	8	6
50–60	262	118	73	117	58	25	19
>60	230	116	82	85	59	33	29
Total No. of males	580	286	177	239	128	66	54

well as the mean ages of the case subjects and control subjects overall and by sex. The incident cancers included 590 esophageal cancers, 402 gastric cardia cancers, and 87 gastric non-cardia gastric cancers. With the exception of patients with incident esophageal cancers, there were more male than female case subjects. Fifty-six percent (332 of 590) of esophageal cancers, 58% (232 of 402) of gastric cardia cancers, and 78% (68 of 87) of gastric non-cardia cancers were fatal. For each age–sex stratum, the difference (not shown) between the mean age of the case subjects and the mean age of the control subjects was statistically not significant. The largest within-strata age difference occurred in the category of females 50 years old or younger: The mean age of the case subjects was 1.5 years greater than that of the control subjects ($P = .82$ for difference in means). All other age means differed by less than one-half year.

Using the known sample weights and the serum selenium levels of the study population, we estimated the mean value of selenium (Table 2) in the General Population Trial cohort as 72.2 μg/L. Ten percent of the population had selenium values less than 51.3 μg/L; only 10% of the population had values higher than 92.8 μg/L. The overall population quartiles served as the boundaries for the models that expressed the cancer RR in terms of the selenium quartiles. The selenium distribution differed significantly by age–sex strata ($P<.001$). The main source of this variation was the lower selenium values found in females 50 years old or younger (Table 2). The 75th percentile of the females 50 years old or younger (76.0 μg/L) was nearly identical to the 50th percentile of the other two female strata (75.9 μg/L for females 50–60 years old; 74.0 μg/L for females ⩾60 years old), indicating a considerable shift toward lower values among younger women. This difference was not due to the arbitrary nature of the age stratum: Regression models with age as a continuous variable also demonstrated the selenium values to be significantly lower for females in this age range. Among the males, the older males had slightly lower values than the males in the other two age strata. Once the females 50 years old or younger were accounted for, the differences between the other five strata were not significant ($P = .11$). The differences in selenium levels by smoking, alcohol consumption, or randomization to factor D (containing 50 μg of yeast selenium) were small and statistically not significant (*see* Table 2 for *P* values).

Combined over all three of the cancer sites, the mean serum selenium level in the control subjects, 72.3 μg/L, was approximately 4% higher than that in the case subjects, 69.5 μg/L

Table 2. Means (standard deviation [SD]) and selected quantiles for the pretrial serum selenium levels (μg/L) in the General Population Trial cohort in Linxian, China: overall* and by age, sex, smoking,† drinking,† and factor D supplementation‡

		Selenium level, μg/L					
	No.	Mean (SD)	10%	25%	50%	75%	90%
Overall	2141	72.2 (0.57)	51.3	59.8	70.9	82.1	92.8
⩽50 y	444	69.8 (1.03)	51.6	58.7	67.8	78.5	89.7
50–60 y	939	75.2 (0.80)	52.0	62.2	74.2	85.7	97.1
>60 y	758	72.6 (0.83)	49.9	60.4	72.2	83.4	93.0
Female§	970	71.0 (0.82)	50.2	57.7	69.9	81.3	92.1
⩽50 y	259	66.7 (1.38)	47.9	55.9	65.1	76.0	85.1
50–60 y	417	75.5 (1.22)	50.9	60.3	75.9	87.1	98.0
>60 y	294	73.9 (1.29)	52.3	62.5	74.0	85.2	92.8
Male§	1171	73.8 (0.77)	53.5	63.1	71.6	83.3	94.4
⩽50 y	185	74.2 (1.54)	56.6	64.8	71.4	83.4	93.1
50–60 y	522	74.9 (0.96)	53.0	64.2	73.2	84.4	96.3
>60 y	464	71.3 (1.04)	47.7	59.9	69.9	81.2	93.6
Smoking¶							
No	1300	71.9 (0.72)	50.8	58.7	71.1	82.0	92.8
Yes	838	73.1 (0.89)	53.2	62.4	70.7	82.3	93.1
Drinking¶							
No	457	71.7 (0.63)	50.7	59.1	70.2	81.6	92.9
Yes	1681	74.7 (1.28)	54.2	65.1	75.1	84.7	92.7
Factor D¶							
No	1077	71.4 (0.82)	50.0	60.0	70.0	81.7	91.7
Yes	1064	73.0 (0.81)	52.5	59.6	71.7	82.4	93.6

*Selenium means (SDs) and quartiles were calculated with the use of the sample weights of the case subjects and control subjects selected for the serum study. These means and quartiles estimate the selenium distribution in the entire General Population Trial cohort of 29 584. The quartile values of the overall population (*see* row 1: 25%, 50%, and 75%) were used as the boundaries for the analyses based on quartiles. The overall 10th percentile was used as the upper limit of the boundary for "low" selenium. When selenium is used as a continuous variable, the relative risks are given in the unit of 11 μg/L, which is a rounded estimate of the average quartile size of the two central quartiles (82.1 – 59.8)/2 = 11.15.

†Smoking: lifetime use of cigarettes for 6 or more months. Drinking: ever drinking alcoholic beverages in the last 12 months. Three male subjects had missing information on baseline smoking and drinking status.

‡Factor D was the supplement that contained 50 μg of yeast selenium, 15 mg of β-carotene, and 30 mg of α-tocopherol.

§There were significant differences in selenium values by age and sex strata ($P<.001$); the main source of this variation was the lower selenium values in the females 50 years old or younger. *See* text for more discussion.

¶Smoking and alcohol consumption were highly correlated with sex. In this study, 71.6% of the males smoked. Only two females (0.2%) smoked; 33.6% of males and 6.6% of females consumed alcohol. Male smokers did not have statistically significant higher means ($P = .26$) than nonsmokers. Neither male drinkers (difference in means = 1.12; $P = .50$) nor female drinkers (difference in means = 1.64; $P = .10$) had statistically significant higher selenium levels than the corresponding nondrinkers. There was no significant difference ($P = .16$) in the pretrial selenium levels among those who did and did not receive factor D.

Table 3. Mean (standard deviation [SD]) and median serum selenium levels* (μg/L) among control subjects and case subjects by cancer type in the General Population Trial cohort, Linxian, China

	No.	Selenium level, μg/L		Mean difference, mean control subjects − mean case subjects (SD)	P†
		Mean (SD)	Median		
Control subjects	1062	72.3 (0.59)	71.0	—	—
Case subjects					
Cancer incidence					
Combined‡	1079	69.5 (0.15)	67.7	2.8 (0.61)	$<10^{-5}$
Esophageal	590	69.6 (0.19)	67.6	2.7 (0.62)	$<10^{-5}$
Gastric cardia	402	68.4 (0.25)	67.0	3.9 (0.64)	$<10^{-6}$
Gastric non-cardia	87	73.3 (0.66)	70.6	−0.94 (0.88)	.29
Cancer mortality					
Combined‡	632	70.2 (0.20)	68.3	2.2 (0.62)	$<10^{-3}$
Esophageal	332	70.0 (0.27)	67.5	2.3 (0.65)	$<10^{-3}$
Gastric cardia	232	69.4 (0.31)	68.7	2.9 (0.67)	$<10^{-4}$
Gastric non-cardia	68	73.3 (0.79)	70.6	−1.02 (0.99)	.30

*The means (SDs) and medians for each age control category were obtained with the use of the sex and age strata sampling weights of the individuals.
†*P* values are based on a Student's *t* test comparing the difference in the mean of the control subjects with the mean of the case subjects.
‡Combined category includes esophageal and gastric cardia and non-cardia cancers.

($P<10^{-5}$; Table 3). This overall difference was attributable to the lower mean selenium values in the subjects with incident esophageal (69.6 μg/L) and gastric cardia (68.4 μg/L) cancers. The subjects with gastric non-cardia cancers had statistically not significant higher mean selenium values (73.3 μg/L) than the control subjects. The same pattern existed when the analyses were restricted to fatal cancers (Table 3).

Table 4 presents the RRs, 95% CIs, and tests of statistical significance relating serum selenium levels to the subsequent development of site-specific cancers. We present the RRs by using two different exposure measures: 1) selenium as a continuous variable (each unit = 11.0 μg/L) and 2) selenium values classified into population quartiles. In terms of the magnitude of RR, the dose–response relationship, and the tests of statistical significance, the differences between these two exposure measures were negligible. The risks for both incident esophageal (*P* for trend $<10^{-4}$) and gastric cardia (*P* for trend $<10^{-6}$) cancers decreased as serum selenium levels increased. Compared with individuals in the lowest quartile of selenium, those in the highest quartiles had a 44% reduction (RR = 0.56; 95% CI = 0.44–0.71) in incident esophageal cancer and a 53% reduction (RR = 0.47; 95% CI = 0.33–0.65) in incident gastric cardia cancer. The RRs for both cancers declined monotonically with each increasing quartile. For incident esophageal cancer, this pattern is consistent with a linear decrease with increasing selenium levels ($P = .81$ for test for nonlinearity). For incident cancer of the gastric cardia, the pattern suggested a superlinear (quadratic) decrease ($P = .06$), which was not borne out when we restricted the analysis to fatal cancers ($P = .39$). To look for a threshold effect at the low end of the selenium distribution, we allowed those in the lowest population decile (selenium <51.3 μg/L) to have a risk different from that assigned by the linear model. For neither incident esophageal ($P = .97$) nor gastric cardia ($P = .38$) cancers was there any evidence of such a threshold. Setting the selenium threshold at the population 1 percentile value of 36.0 μg/L, which is near the value where clinical symptoms of selenium deficiency can develop *(6,47),* yielded essentially identical results. In contrast, we found no association between serum selenium levels and incident gastric non-cardia cancer (*P* for trend = .96). When restricted to fatal

Table 4. Relative risks* (RRs) and 95% confidence intervals (CIs) for changes in serum selenium levels (per 11.0 μg/L) and selenium quartiles by cancer site in the General Population Trial cohort, Linxian, China

	Selenium per 11.0 μg/L			Selenium quartiles†							
				1	2		3		4 (highest)		
	RR	95% CI	P	(lowest)	RR	95% CI	RR	95% CI	RR	95% CI	P for trend‡
Cancer incidence											
Esophageal	0.89	0.83–0.95	$<10^{-3}$	1	0.84	0.66–1.07	0.66	0.52–0.83	0.56	0.44–0.71	$<10^{-4}$
Gastric cardia	0.83	0.77–0.90	$<10^{-5}$	1	0.75	0.55–1.03	0.55	0.40–0.77	0.47	0.33–0.65	$<10^{-6}$
Gastric non-cardia	1.02	0.89–1.18	.75	1	1.20	0.62–2.29	1.08	0.56–2.06	1.07	0.55–2.08	.96
Cancer mortality											
Esophageal	0.90	0.83–0.97	<.01	1	0.92	0.66–1.30	0.66	0.46–0.93	0.62	0.44–0.89	.02
Gastric cardia	0.87	0.79–0.96	<.01	1	0.89	0.59–1.32	0.76	0.51–1.13	0.59	0.39–0.90	.01
Gastric non-cardia	1.02	0.88–1.20	.77	1	1.06	0.51–2.19	0.95	0.46–2.00	1.03	0.85–2.02	.98

*RR, 95% CIs, and *P* values come from regression models stratified on sex and age. Additional age adjustment is provided by a continuous age term unique to each stratum.
†The boundaries for the quartiles are given in Table 2. The within-quartile medians from low to high are 52, 66, 76, and 92 μg/L, respectively.
‡*P* value for trend, frequently referred to as a trend test, tests the hypothesis that a model in which each individual is assigned the selenium values equal to his/her quartile number is superior to a model in which no selenium values are entered. Tests performed for detecting deviations from the linear (on the log scale) decrease in RR specified by the continuous selenium and quartile trend models failed to detect significant deviations. *See* text for details.

cancers only, the RRs for all three cancer sites were similar to those for the incident cancers (Table 4).

The specification of the models generating the RR estimates from Table 4 assumed that the effect of selenium on cancer rates did not vary by sex, age, or time between blood draw and the development of cancer. We examined whether the RR of selenium varied within these categories, as well as within categories defined by factor D, smoking, and alcohol consumption (Table 5). We found no evidence that sex affected the RR of selenium (P>.60 for all cancer sites). For esophageal and gastric cardia cancers, which showed an inverse association with selenium, the RR estimates for males and females were nearly identical (Table 5). Similarly, RRs did not differ by age strata for any of the cancers (not shown). To address the concern that occult preclinical cancers might lower selenium levels and contribute to the association with cancer rates, we examined whether the RR associated with cancer that developed in the first 2 years of the study (3 years after blood draw) differed from the RR associated with cancer that developed after 2 years. For both esophageal and gastric cardia cancers, the association of higher selenium levels with lower cancer incidence was stronger for the cancers that occurred after 2 years (Table 5). In neither case were these differences significant (P = .18 for esophageal cancer; P = .44 for gastric cardia cancer).

One year after providing the serum on which these selenium measurement were made, approximately half of the 2141 persons in this study began taking 50 μg of supplemental selenium in factor D (Table 2). As expected, we found the same overall beneficial effect of factor D on cancer outcomes (not shown) as we reported in the original analyses of the trial *(8):* The factor D group had lower RRs in terms of incidence of and mortality from the cancers of the three sites combined. However, we found no evidence that the selenium in factor D modified the association between preintervention selenium levels and cancer risk (all P values >.20; Table 5): For esophageal and gastric cardia cancers, the inverse association was the same, regardless of whether factor D was taken. For gastric non-cardia cancer (P = .62), there was no evidence of an association in either factor D group.

Smokers had an RR of esophageal cancer 1.5 times that of nonsmokers (RR = 1.5; 95% CI = 1.10–2.10), but no excess was seen for either gastric cardia or non-cardia cancers. Alcohol consumption was not a risk factor for cancer of any of the sites; moreover, there was not a statistically significant alcohol consumption and smoking interaction. These results were the same as those previously reported in an analysis of the entire cohort *(48).* Neither smoking nor alcohol consumption modified the effect of selenium on the risk of esophageal or gastric cardia cancer (Table 5).

Fig. 1 and Table 6 show the effects of serum selenium levels on absolute cancer risk. For esophageal cancer (Fig. 1, A) and gastric cardia cancer (Fig. 1, B), there was a clear stepwise separation of cumulative incidence curves by quartile of selenium. This stepwise separation corresponds to the monotonic decrease in RR with rising selenium quartiles reported in Table 4. For gastric non-cardia cancer (Fig. 1, B), there was a much smaller overall incidence as well as no statistically significant differences in incidence by selenium quartile. In Fig. 1, C, we grouped esophageal and gastric cardia cancers into one category. Just over 5% of the people in quartile 1 developed one of these two cancers in the 5.25 years of follow-up. This cumulative incidence was 2.35% greater (P<10^{-5}) (Table 6) than that for persons in the highest quartile of serum selenium. For each cancer site, we calculated the population attributable risk as the percentage of the total cancers in this population that would not occur if individuals with selenium levels in the lowest

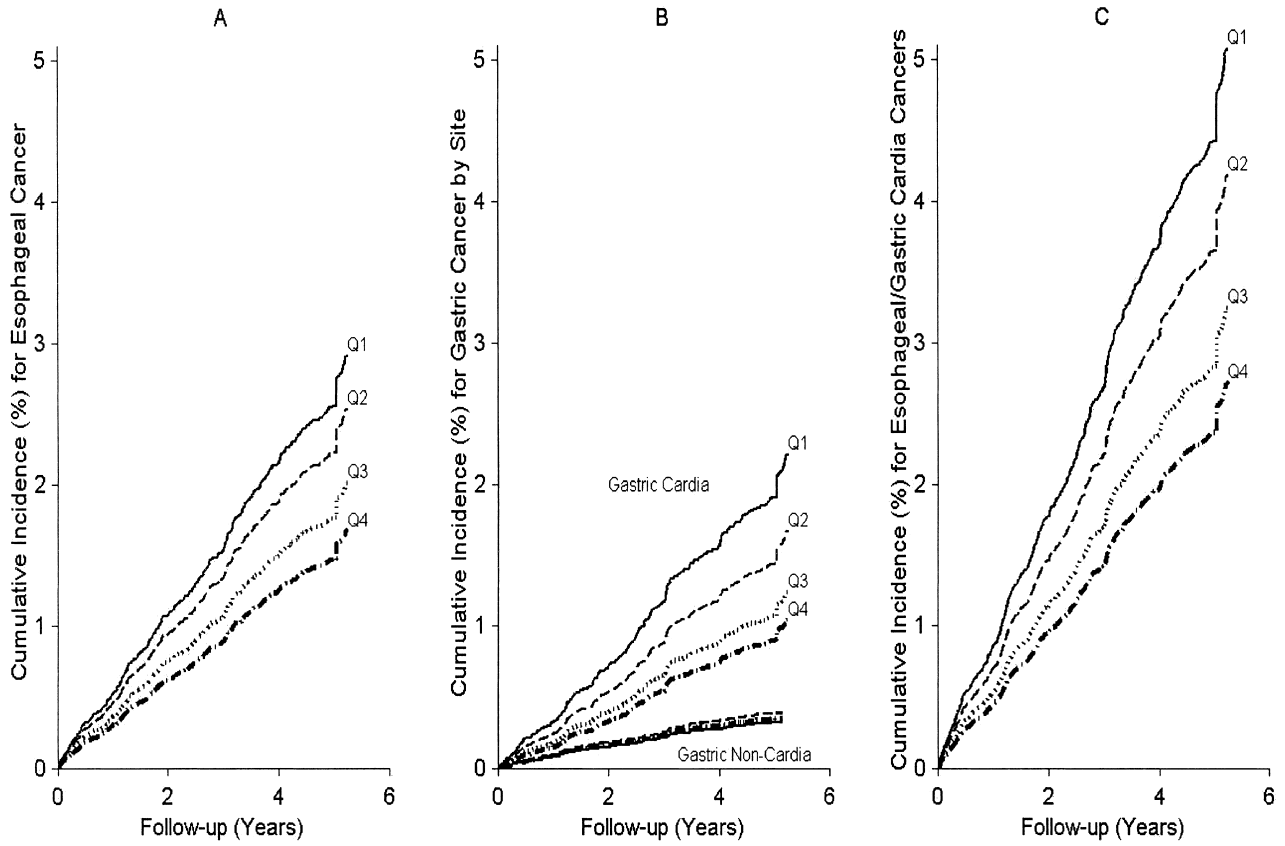
Table 5. Relative risks* (RRs) and 95% confidence intervals (CIs) of serum selenium levels (per 11.0 μg/L) within categories defined by sex, time to cancer, factor D, smoking, and alcohol consumption for site-specific cancer incidence in the General Population Trial cohort, Linxian, China

	Esophageal cancer			Gastric cardia cancer			Gastric non-cardia cancer		
	RR	95% CI	P	RR	95% CI	P	RR	95% CI	P
Sex									
Females	0.89	0.81–0.98	.84	0.82	0.73–0.92	.71	0.91	0.68–1.21	.62
Males	0.88	0.80–0.97		0.84	0.76–0.93		1.06	0.90–1.24	
Time to cancer, y†									
<2	0.94	0.85–1.03	.18	0.87	0.77–0.97	.44	1.04	0.86–1.26	.78
⩾2	0.86	0.78–0.94		0.81	0.74–0.90		1.00	0.82–1.23	
Factor D									
No	0.91	0.83–1.00	.46	0.87	0.78–0.96	.24	1.05	0.88–1.26	.62
Yes	0.87	0.79–0.95		0.79	0.70–0.89		0.98	0.79–1.22	
Smoking‡									
No	0.91	0.75–1.09	.72	0.83	0.69–1.01	.95	0.85	0.63–1.16	.09
Yes	0.87	0.78–0.98		0.83	0.73–0.94		1.16	0.96–1.41	
Alcohol consumption									
No	0.88	0.78–0.99	.80	0.82	0.72–0.93	.74	1.03	0.85–1.26	.62
Yes	0.86	0.72–1.01		0.85	0.71–1.03		1.13	0.85–1.51	

*Within each category, the RR estimates for a specific group came from models restricted to individuals in that group. For example, within the category of sex, males did not contribute to the RR estimate of the effect of selenium on cancer in females. All models were stratified on age and sex, with additional age adjustment provided by a continuous age term unique to each stratum. P values are for tests of the hypothesis that there was no between-group variation in the RRs. Tests compared a model with the main effect of a category and a main effect of selenium with a model that contains the main effect term for the category and separate selenium effects for each group. Since all models were stratified on sex and age, there was no main effect term when testing and estimating the sex interaction.

†Time was measured as time since the commencement of the active intervention. This was 1 year after the drawing of the blood used for the selenium measurements.

‡Smoking models were limited to males.



Cumulative Incidence (%) and 95% Confidence Intervals

	Esophageal			Gastric Cardia			Esophageal and Gastric Cardia		
	2 y	4 y	5.25 y	2 y	4 y	5.25 y	2 y	4 y	5.25 y
Q1	1.08(0.86-1.30)	2.16(1.75-2.56)	2.91(2.39-3.44)	0.72(0.55-0.90)	1.57(1.24-1.91)	2.23(1.77-2.68)	1.80(1.49-2.11)	3.71(3.13-4.28)	5.08(4.32-5.85)
Q2	0.94(0.74-1.14)	1.88(1.52-2.24)	2.54(2.07-3.01)	0.54(0.40-0.68)	1.19(0.92-1.45)	1.68(1.31-2.05)	1.48(1.22-1.75)	3.06(2.56-3.55)	4.20(3.54-4.85)
Q3	0.75(0.59-0.90)	1.49(1.21-1.78)	2.02(1.64-2.40)	0.40(0.30-0.51)	0.88(0.67-1.08)	1.25(0.96-1.53)	1.15(0.94-1.35)	2.37(1.98-2.75)	3.26(2.74-3.77)
Q4	0.62(0.48-0.76)	1.24(0.98-1.51)	1.68(1.33-2.03)	0.34(0.24-0.43)	0.74(0.55-0.93)	1.05(0.78-1.31)	0.96(0.77-1.14)	1.98(1.63-2.34)	2.73(2.25-3.20)

	Gastric Non-Cardia		
	2 y	4 y	5.25 y
Q1	0.15(0.07-0.24)	0.28(0.14-0.42)	0.33(0.17-0.49)
Q2	0.18(0.10-0.27)	0.33(0.19-0.47)	0.39(0.23-0.55)
Q3	0.17(0.09-0.24)	0.30(0.18-0.43)	0.35(0.21-0.50)
Q4	0.16(0.09-0.24)	0.30(0.17-0.43)	0.35(0.20-0.50)

Fig. 1. Cumulative incidence by quartile of serum selenium for cancers of the gastric cardia, the gastric non-cardia, the esophagus, and the esophagus and gastric cardia combined. **Thin solid line** = Q1 (i.e., quartile 1); **thin dashed line** = Q2 (i.e., quartile 2); **thick dotted line** = Q3 (i.e., quartile 3); **thick dotted–dashed line** = Q4 (i.e., quartile 4). Incidence curves were adjusted for the association of selenium levels with age and sex. The abrupt rise in incidence for gastric cardia and esophageal cancers at the end of the study was due to the detection of these cancers by the end of trial examination procedures. *See* Table 6 for numeric details and for *P* values for differences in cancer risk by quartile. **Panel A:** There was a statistically significant difference by quartile of selenium in the cumulative incidence of esophageal cancer. By the end of the study period, 1.23 more cancers developed per 100 people in the lowest than in the highest quartile. **Panel B:** As was the case for esophageal cancer, the incidence of gastric cardia cancer was statistically significantly higher in the lower selenium quartiles. By the end of the study period, there were 1.18 more gastric cardia cancers per 100 person in the lowest quartile than in the highest quartile of this cohort. For gastric non-cardia cancers, a cancer whose overall incidence was considerably less than that of gastric cardia cancers, the incidence curves by selenium quartile were nearly identical. **Panel C:** Esophageal and gastric cardia cancers both occurred in epidemic proportions in this population and historically have not been recorded as distinct diseases. During the study period of 5.25 years of follow-up, slightly more than 5% of individuals in the lowest selenium quartile developed one of these two cancers. In contrast, these two cancers occurred in only 2.7% of those in the highest quartile of selenium. **Table under the cumulative incidence curves** gives the cumulative incidence and 95% confidence intervals for each quartile at the selected follow-up times of 2 years, 4 years, and 5.25 years (end of study).

three quartiles had the cancer rate of individuals in the highest quartile. For esophageal/gastric cardia cancers, we estimated that such a risk change would eliminate 26.4% (95% CI = 14.45–38.36) of these cancers. When restricting the analysis to fatal cancers, we found a similar population attributable risk (Table 6).

Table 6. Absolute risks, risk differences, and the population attributable risks* for incidence of and mortality from esophageal and gastric cancers in the General Population Trial cohort, Linxian, China

	Cumulative incidence at 5.25 y		Difference in cumulative incidence: quartile 1 − quartile 4 (95% confidence interval)	P†	Population attributable risk (95% confidence interval)
	Quartile 1	Quartile 4			
Cancer incidence					
Esophageal/gastric cardia‡	5.08%	2.73%	2.35% (1.60 to 3.10)	$<10^{-5}$	26.4% (14.45 to 38.36)
Esophageal	2.91%	1.68%	1.23% (0.73 to 1.73)	$<10^{-4}$	26.5% (11.90 to 41.12)
Gastric cardia	2.23%	1.05%	1.18% (0.81 to 1.55)	$<10^{-5}$	32.5% (16.43 to 48.59)
Gastric non-cardia	0.33%	0.35%	−0.023% (−0.26 to 0.21)	0.85	1.43% (−36.25 to 39.13)
Cancer mortality					
Esophageal/gastric cardia‡	2.67%	1.64%	1.04% (0.49 to 1.59)	$<10^{-3}$	21.1% (6.33 to 35.83)
Esophageal	1.62%	1.01%	0.61% (0.24 to 0.989)	.001	22.1% (3.41 to 40.79)
Gastric cardia	1.05%	0.62%	0.43% (0.16 to 0.69)	.002	26.8% (5.78 to 47.77)
Gastric non-cardia	0.29%	0.30%	−0.009% (−0.22 to 0.20)	.93	−2.4% (−45.10 to 40.40)

*The population attributable risk is the percentage of cancers in this population that would not occur if individuals with selenium levels in the lowest three quartiles had the cancer rate of individuals in the highest selenium quartile.

†P values are for the difference in cumulative incidence and are based on the normal distribution.

‡Includes cancers of the esophagus and of the gastric cardia.

Discussion

Our study of nearly 1100 incident cases of cancer is one of the largest prospective studies of serum selenium levels and cancer risk and has more site-specific cancers than any previous study. We found highly significant ($P<10^{-4}$) inverse associations of serum selenium levels with the incidence of both esophageal and gastric cardia cancers over a period of 5.25 years of follow-up. Individuals in the highest quartile of selenium developed these cancers at approximately half the rate as individuals in the lowest quartile. For both cancers, there was a dose–response relationship: The log RR decreased linearly with increasing selenium levels. For neither cancer was there evidence for a low threshold beneath which superlinear risks were evident, nor was there evidence that the change in RR was sublinear near the upper end of the selenium distribution. In general, the RRs were identical for men and for women and did not vary by age (Table 5). The main trial results found that supplementation with the selenium-containing factor D reduced the absolute risk of cancer *(8).* In this serologic study, we found that the increased RRs associated with low selenium levels existed regardless of whether individuals did or did not receive supplemental selenium, indicating that this dose of supplement does not immediately attenuate the biologic consequences of presumably long-term low levels of selenium. Although we have no direct measurements of the average increase in selenium levels in those who received factor D, a linear interpolation from the increases seen in the trial by Clark et al. *(6)* predicts a mean increase of 19.0 μg/L. This is approximately the increase needed to raise a person from the first to the third quartile. These findings were essentially unchanged when we restricted the analyses to the fatal cases only.

In Linxian, esophageal and gastric cardia cancers account for approximately 25% of all deaths *(49).* As demonstrated in Fig. 1, classifying individuals by selenium quartile partitions this risk into distinct groups: The cumulative incidence of esophageal/gastric cardia cancers was 5.01% for individuals in the lowest quartile of selenium and 2.7% for those in the highest quartile. If altering serum selenium levels would correspondingly change the cancer rates, then 26.4% (95% CI = 14.45–38.36) of the epidemic of esophageal/gastric cardia cancers is attributable to low selenium levels. Since, by necessity, we have assumed that persons in the fourth quartile of selenium, where the median selenium level is 92.0 μg/L, would receive no benefit from higher levels of serum selenium, this may be an underestimate of the population attributable risk. Our analyses of the dose–response relationship and the results from the study by Clark et al. *(6)* suggest that even individuals in the fourth quartile would benefit from higher values of selenium.

In contrast, we found no convincing evidence for an inverse association between serum selenium levels and the incidence of gastric non-cardia cancers. The mean selenium values for subjects with gastric non-cardia cancers were insignificantly higher than those for control subjects (Table 3), and the RR at the highest three quartiles of selenium was insignificantly greater than that at the lowest quartile (Table 4). The only suggestion of a relationship between serum selenium levels and gastric non-cardia cancer incidence occurred in the subgroup of male nonsmokers, with those in the upper three quartiles of selenium having a lower RR than those in the lowest quartile (data not shown). Given the number of subgroups explored, the lack of an association in female nonsmokers, and the absence of either a sex or smoking interaction for esophageal and gastric cardia cancers, this finding may have resulted by chance.

With the exception of one study reporting nine cases of esophageal cancer *(12),* there have been no prospective studies evaluating the association of serum selenium levels with the occurrence of esophageal cancer or gastric cancers by subsite, although several studies *(13–18)* have investigated gastric cancer overall. Since there is considerable evidence from the prospective studies of selenium and lung cancer that associations depend on the selenium levels of the population, it is noteworthy that the mean population selenium value of our cohort (72.2 μg/L) was low when compared with that of populations of most Western countries. Four studies of two separate Finnish cohorts *(12–15)* reported similarly low mean levels of selenium, ranging between 55 and 65 μg/L. One of these cohorts *(12–14)* contained nine case subjects with esophageal cancer *(12)* and found an insignificantly lower ($P = .10$) selenium level in case subjects (60.4 μg/L) than in control subjects (72.0 μg/L). The only statistically significant relationship between the occurrence of stomach cancer and the level of selenium was reported in the 10-year follow-up *(14)* from this cohort: Both male (n = 38) and

female (n = 37) case subjects with stomach cancer had lower serum selenium levels than control subjects, with statistically significant differences only among males. For both sexes, the RR was lowest in the highest quintile of selenium. The inverse association of higher selenium levels (top versus bottom quintile) with lower cancer rates was greatest in men (RR = 0.09) but was also evident in women (RR = 0.27). In the earlier report from that cohort *(13)* with 7 years of follow-up, the esophageal and gastric cancers were grouped together (n = 87), and the findings were similar. The other Finnish cohort *(15)* contained 11 patients with gastric cancers and found no difference in the mean selenium levels of case subjects versus control subjects. A cohort study *(16)* from The Netherlands, where selenium levels were moderate *(19,20),* found no difference in mean selenium levels between control subjects and male (n = 80) or female (n = 20) stomach cancer patients. In males, the cancer risk was lowest in the highest quintile of selenium (RR = 0.40 [95% CI = 0.33–1.27]), but the trend was nonmonotonic (the fourth quintile had higher cancer rates than the second and third) and not statistically significant.

Outside our study, the largest study of stomach cancers (n = 202) comes from a cohort of atomic bomb survivors from Japan *(17),* where the mean selenium level was 119 μg/L, with the lowest quartile consisting of individuals with selenium levels lower than 99 μg/L. There was no evidence of an association: The RR of stomach cancer from highest to lowest quartile was 1. Similarly, a study of 66 case subjects with stomach cancer among men of Japanese ancestry in Hawaii *(18)* found no association between the occurrence of stomach cancer and serum selenium levels. In this group, the mean selenium level was 125.0 μg/L, with the lowest quintile composed of persons with selenium levels lower than 103.1 μg/L.

A large number of prospective studies have examined the association of selenium levels with the incidence of total cancer *(20–22,24–27)* and cancers of the lung *(17–19,22,24,28–31),* prostate *(21,25,35,50),* gastrointestinal tract *(21,22,24,26),* colon *(32),* pancreas *(33),* liver *(23),* or female organs *(51,52).* For lung cancer, the inverse associations have been most pronounced in populations with generally low selenium levels, such as our population *(22,24,29).* The sole exception is a cohort study of tin miners in China *(31),* where no association was found. In populations with higher selenium levels, results have been inconsistent, ranging from statistically significant inverse associations *(19),* inverse associations that do not attain statistical significance *(17,28),* and no association *(18).*

The most direct evidence linking selenium intake to cancer comes from the three clinical trials where individuals were randomly assigned to receive either a selenium-containing supplement or a placebo *(6–9,11).* In our cohort, the individuals who received factor D (50 μg of yeast selenium, 15 mg of β-carotene, and 30 mg of α-tocopherol) had statistically significant reductions in overall mortality and in cancer mortality, with the largest reduction seen for gastric cancer mortality, including gastric cardia cancers (RR = 0.79; 95% CI = 0.64–0.99) and noncardia cancers (RR = 0.72; 95% CI = 0.46–1.14). Little effect was seen for esophageal cancer (RR = 0.96; 95% CI = 0.78–1.18).

However, in the smaller Linxian trial in which a multivitamin with 50 μg of sodium selenate was the active agent *(9),* the largest site-specific reduction in mortality occurred for esophageal cancer (RR = 0.84; 95% CI = 0.54–1.29). In the U.S. randomized trial *(6),* there was a total of eight esophageal cancer cases: Two occurred in the treated group, and six occurred in the group given the placebo (RR = 0.33; 95% CI = 0.03–1.84).

Given the large number of site-specific cancers, the close follow-up of the individuals in the cohort, and the rigorous documentation of the cancer diagnoses *(8,9),* neither chance nor disease misclassification is a viable explanation for the inverse relationship observed between selenium levels and the risk of esophageal and gastric cardia cancers. There is always concern that preclinical disease can alter serum measurements and thus can create a misleading association. By the design of the General Population Trial, all events that occurred within the first year after the blood was drawn were excluded. When we further separated the cancers in the next 2 years from those in the last 3.25 years, we found that the effects in these later years were slightly greater than those in the earlier time period. Overall, the differences by interval were not statistically significant, which is consistent with previous studies *(13–15,18,21–23,28,30,33,34,52).* Unlike Western countries where smoking and alcohol consumption contribute substantially to esophageal cancers risk *(53)* and may confound associations with selenium levels, these exposures have had only a minor impact on esophageal cancer in this population *(48)* and no effect on the cancer–selenium relationship (Table 5). Age and sex, which are associated with both population selenium levels and population cancer rates, were controlled for in the design of the study by frequency matching and in the analyses by using Cox RR models stratified on age–sex with additional stratum-specific age terms used to adjust for residual within-stratum variation.

Unmeasured confounders are always a possible explanation for an association in any observational study. From pretrial measurements, we know that Linxian residents had low levels of a number of vitamins. Hence, the associations with selenium may be due to another nutrient that covaries with selenium. While prior studies in which several other vitamins were measured vary with respect to their effect modification, none have found that controlling for these vitamins modifies the estimated main effect of selenium *(13,18,19,21,23,24,28–30,32–34,50,54).* We are currently completing measurements on a number of fat-soluble and water-soluble vitamins to determine their impact on these cancers and to see whether they modulate the selenium effect. The major limitation of our study and of other prospective studies of selenium is that the exact sources for the variation in selenium levels are unknown. Although it is clear that increases in dietary selenium elevate serum levels and selenium-dependent enzyme activity *(20,34,47,50,55),* there is only a weak association between serum selenium levels and selenium levels assessed by typical dietary records *(47).* Thus, we cannot exclude the possibility that the variations in selenium levels may be influenced by environmental or genetic influences whose effects on selenium levels are incidental to their effects on cancer rates. In other populations where elaborate dietary records were gathered and a locally accurate selenium food database was compiled, differences in selenium intake were highly correlated (correlation coefficients >.8) with variation in serum levels *(47).*

Our study adds support to the already considerable body of evidence that variation in selenium levels affects the incidence of human cancers. In Linxian, where there is an epidemic of esophageal/gastric cardia cancers accompanied by low selenium levels, it is important to decide whether selenium supplementation, either alone or in conjunction with other nutrients, should

begin at the population level. During the next 12 months, we expect additional pertinent information to emerge from our evaluations of serum vitamins, from analyses of the post-trial mortality and cancer incidence experience, and from a recently completed nutritional study on 1000 cohort members.

Greater uncertainties are involved in drawing inference from this population in China to high-risk populations in the United States for squamous esophageal cancer *(56,57)* or for adenocarcinomas of the gastric cardia and distal esophagus *(56,57).* In general, the descriptive features and risk factors for squamous esophageal cancer differ between populations with epidemic rates such as Linxian and Western populations *(53).* Similarly, some of the risk factors associated with the adenocarcinomas of the esophagus and gastric cardia, such as obesity, reflux disease, and Barrett's metaplasia *(56,58–60),* are virtually absent in Linxian *(48,61).* Nonetheless, discrepancies in clinical and demographic characteristics need not imply differences in biologic pathways. The importance of selenium enzymes in the prevention of and response to tissue damage *(1–5)* may place selenium at the crossroads of various exposure–cancer relationships. On the basis of prospective studies and the intervention trial of Clark et al. *(6),* it has been suggested that larger randomized trials will be required to evaluate the potential protective effects of selenium on lung and prostate cancers and that these trials should be sufficiently large and inclusive enough to evaluate other end points as well *(62).* In particular, our findings indicate the need to include populations at high risk for squamous cell carcinoma of the esophagus and adenocarcinoma of the gastric cardia in future trials.

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NOTES

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